Morphology and Histology of the Alimentary Canal of *Lygus hesperus* (Heteroptera: Cimicomorpha: Miridae)

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ABSTRACT Microdissection and transverse semithin sections were used to perform a light microscopy survey of the gross morphology and cellular anatomy of the alimentary canal, respectively, of *Lygus hesperus* Knight, a key pest of cotton (*Gossypium hirsutum* L.), alfalfa (*Medicago sativa* L.), and other crops. The gross morphology of the alimentary canal showed a relatively unadorned tube compared with other hemipterans, with variably shaped compartments and one small diverticulum. However, the epithelial cell anatomy of the gut was relatively complex, with the midgut having the most diverse structure and cell types. The midgut was typical of the "*Lygus*-type gut" seen in the older literature, i.e., it consisted of three major regions, the first (descending), second (ascending), and third (descending) ventriculi, with different variants of three major epithelial cell types in each region. Our light microscopy (LM) study suggests that the three cell types are nondifferentiated regenerative cells (which sparsely occurred throughout the midgut but were abundant in the anterior region of the first ventriculus), endocrine cells, and columnar cells. Although the *Lygus* gut cells strongly resemble those cell types seen in other insects, their identification should be confirmed via transmission electron microscopy to be considered definitive. These cell types differed in the size and opacity of vesicles, geometry of cell surface in the gut lumen, and size, shape, and concentration of brush-border microvilli and location within the gut. Comparison of gut structure in *L. hesperus* with that of other hemipterans, especially in relation to hemipteran phylogeny and feeding strategies, is discussed.

KEY WORDS light microscopy, histology, morphology, insect midgut, columnar epithelial cells

The order Hemiptera contains numerous beneficial insects (Deitz et al. 1976) and agricultural pests (Schaefer and Panizzi 2000). The pest species damage agricultural crops either indirectly (by transmitting phytopathogens) or by direct feeding damage mediated by saliva, stylet wounding, or a combination. One of the most notorious direct-damaging pests is *Lygus hesperus* Knight, a phytophagous, piercing–sucking insect that attacks 117 noncrop plants and 25 cultivated crops, such as cotton (*Gossypium hirsutum* L.) and alfalfa (*Medicago sativa* L.) (Schwartz and Foottit 1998, Jones and Jackson 1990). Additionally, it is reasonable to be concerned that *L. hesperus* will become an even more important pest as other previously serious pests, e.g., lepidopteran and coleopteran pests, are managed by *Bacillus thuringiensis* crops and eradication methods.

In spite of the importance of *L. hesperus* feeding, relatively little is known about the cellular anatomy of its alimentary canal. Extensive drawings of the gross gut morphology of representative species of suborder Heteroptera are available (Miyamoto 1961, Goodchild 1966). However, almost no micrographs of cellular anatomy have been published, with the exception of poorly resolved binucleate cells in a pentatomid (Yanai and Iga 1956). In contrast, numerous modern studies have documented the gross morphology, cellular structure, and different cell types of the digestive system of lepidopterans, especially larvae (Engelhard et al. 1991, Baldwin and Hakim 1991, Moffett and Koch 1992), including the binding of toxic compounds to the epithelial cells (Jurat-Fuentes and Adang 2001). Also, passage of macromolecules like IgG and peroxidase from the lumen of the gut into the hemolymph has been studied in various insects (Fishman and Zlotkin

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1984, Borovsky et al. 1994, Habibi et al. 2002, Brandt et al. 2004).

We have published several microscopy and biochemistry studies describing the binding of proteinaceous macromolecules within the midgut and the subsequent passage from the lumen of the gut into the hemolymph in *L. hesperus* (Habibi et al. 2000, 2002, Brandt et al. 2004), and the location and pH range over which proteolytic digestion can occur (Knop-Wright et al., 2006). Further interpretation of those results as they relate to protein binding, toxicity, and digestive structure and function could be made if more detailed knowledge of the gut morphology and cellular anatomy were available. Therefore, the purposes of this study were to 1) provide greater detail of the structure and cellular anatomy of the alimentary canal of *L. hesperus;* 2) present higher resolution light micrographs by using modern microtechniques; 3) provide a greater knowledge base for future studies of the fate of nutrient and non-nutrient proteins; and 4) extend understanding of the feeding anatomy of phytophagous, cimicomophan Heteroptera.

Materials and Methods

Insect Rearing and Microdissection for Internal Morphology. *L. hesperus* were reared as described in Habibi et al. (2000, 2002). Females within 1–5 d after adult eclosion were randomly selected from the stock colony, and they were removed from feeding positions on diet packs immediately before mounting. More than 100 insects were used in the course of this study, and the presence of each attribute displayed in this article was confirmed in 10–15 individual insects. Females were mounted individually, dorsally or ventrally, in black wax that had been poured into the wells of a 12-well dissection dish. The wax was gently melted with a warm spatula before inserting the insect's tergite or sternite into the wax. The mounted, live insects were covered with 200 μl of HEPES wash buffer (70 mM sodium chloride, 30 mM HEPES, and 2 mM calcium chloride, pH 7.4) (Fisher, St. Louis, MO). A small hole was made at the end of the abdomen of each insect by gently pulling the ovipositor. An incision was made in both pleura, and the sternite or tergite (depending upon orientation) was removed without disturbing the natural location of the internal organs. The ovaries, fat bodies, and other organs were removed before staining the alimentary canal with 0.05% toluidine blue. After staining, the alimentary canal was rinsed twice with HEPES wash buffer, observed under an Olympus SZX12 microscope, and photographed with an Olympus DP10 digital camera (Hitschfel Instrument, Inc., St. Louis, MO).

Preparation of Semithin Transverse Sections for LM. The head and thorax together were separated from the abdomen of adult female insects before fixing the tissue in 2% glutaraldehyde and 2% paraformaldehyde in 0.1% cacodylate buffer. After fixation, the samples were rinsed three times in buffer, and postfixed in 1% osmium tetroxide in the same buffer. Samples were subsequently rinsed for 60 min (3 × 20 min) with ultrapure water. Tertiary fixation was done in 1% aqueous uranyl acetate, followed by rinsing for 60 min (3 × 20 min) with ultrapure water. The samples were dehydrated in an ethanol series and infiltrated with Epon/Spurrs resin. The resin was polymerized at 55°C for 2 d, after which the blocks were stored in a desiccator until sectioned.

One-micrometer, semithin sections were cut with an ultramicrotome (Reichert Ultra Cut S, Leica, Wien, Austria). Serial sections were placed on silane-coated slides and stained with 0.5% acid fuchsin and 0.5% toluidine blue. The slides were air-dried at room temperature, mounted with Permount, and viewed with an Olympus (Melville, NY) or Nikon (Melville, NY) microscope. The images were captured using Image-Pro (Media Cybernetics, Inc., Bethesda, MD) and Spot (Diagnostic Instruments, Inc., Sterling Heights, MI) software and further edited for labeling and contrast improvement using Adobe Photoshop 6.0 software (Adobe Systems, Mountain View, CA).

Results

Alimentary Canal Morphology. The ventral view of the alimentary canal showed that the digestive system of *L. hesperus* consisted of a straightforward, unadorned foregut, midgut, Malpighian tubules, and hindgut (Fig. 1). Similar to Yanai and Iga (1956), we found that the midgut was divided into three regions (termed m_1, m_2, and m_3 in their article) and lacked gastric caecae. Yanai and Iga (1956) named this type of heteropteran gut the "Lygus gut." Our transverse sections revealed the internal morphology of the gut regions, and that differing structures denoted further subregions of the alimentary canal. These regions and subregions will be described in their observed order, from anterior to posterior.

Foregut. The foregut was located above the thoracic ganglion between the two flight muscles in the thorax. It was white, 0.4 mm in length and 40 μm in diameter (Fig. 1A and B).

The most anterior part of the foregut, the precibarium, was clearly evident, distinguishable by its proximity to the neuron cell bodies and axon bundles of the precibarial chemosensilla (Fig. 2A). The precibarium was oval in cross section (≈78 μm in diameter) and composed of a thin epithelium in most areas, but also muscles and precibarial sensillar bodies, attached to a thick cuticular wall (Fig. 2A). The cibarium, located posteriad of the precibarium, was arrowhead-shaped in cross-section (≈74 μm in diameter), and also was composed of a thick cuticular wall (Fig. 2B). Two large hammer-shaped cibarial dilator muscles were attached dorsally to the cibarium (Fig. 2C).

The cross-sectional shape of the esophagus (the portion of the foregut posterior to the cibarium) was oval (70 by 35 μm), and the thickened intima observed in the proximal esophagus (Fig. 2D) became thinner as the sections progressed distally. The esophageal sections became circular in shape and wider in diameter (0.165 mm) posteriorly (Fig. 2E), where the

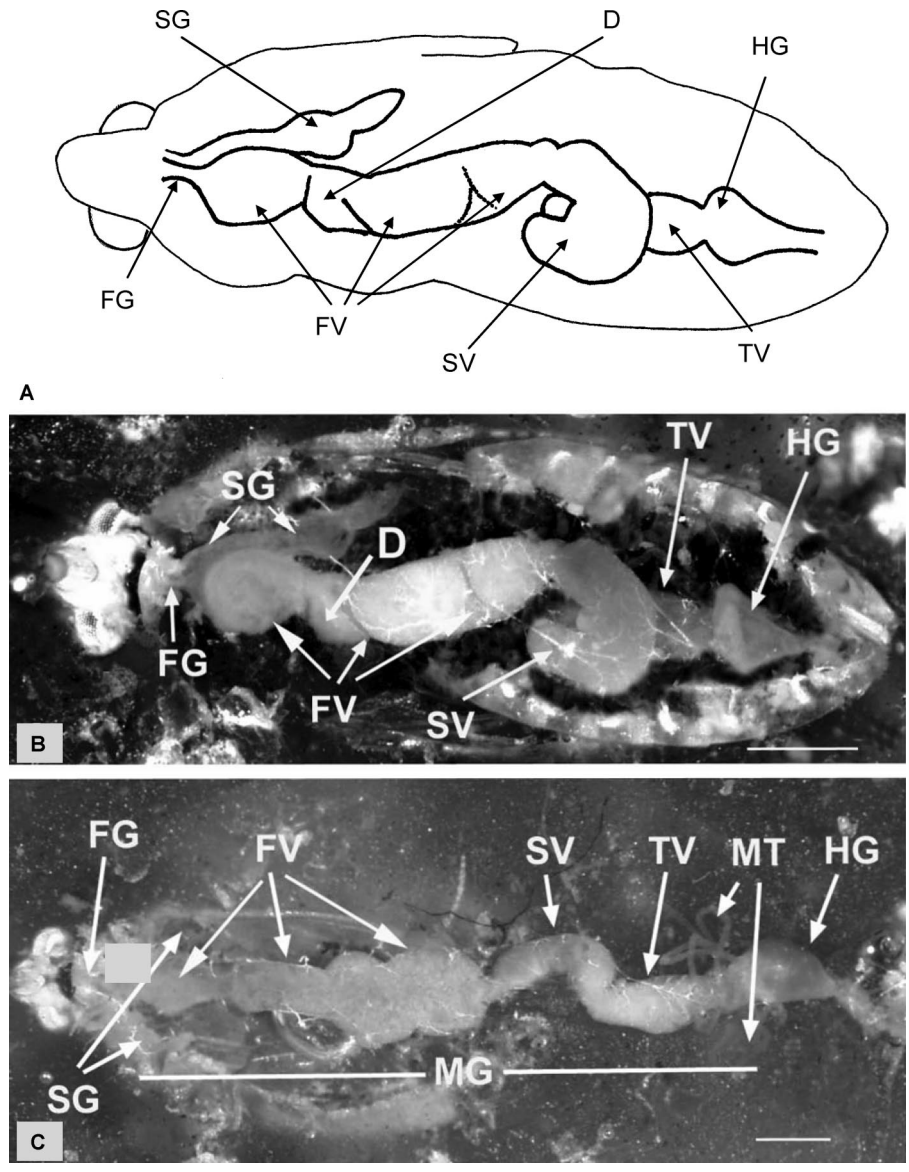

Fig. 1. (A) Interpretive drawing of *L. hesperus* alimentary canal view in B. (B) Ventral view of a partially unfolded alimentary canal. (C) Ventral view of a fully unfolded alimentary canal. The following are identified: short, thin foregut, salivary glands, long first ventriculus, short curved second ventriculus, short third ventriculus, cone-shaped hindgut, and Malpighian tubules. Scale bars = 1 mm. D, diverticulum; FG, foregut; FV, first ventriculus; HG, hindgut; MG, midgut; MT, Malpighian tubules; SG, salivary glands; SV, second ventriculus; TV, third ventriculus.

esophagus joined the midgut. The muscular stomodeal valve, located immediately after the cibarium, was triangular-shaped with two protuberances projecting ventrally into the lumen (Fig. 2F).

Midgut. The long axis of the first ventriculus (m_1 in Yanai and Iga 1956) was aligned dorsoventrally within the thorax. The first ventriculus was located ventrad to the salivary glands within the mesothorax. The most anterior portion of the first ventriculus was composed of two distinct structural formations: a spherical region (0.9–1 mm in length and 0.75 mm in diameter) followed by a narrowed and flattened region (≈0.4 mm in length) (Fig. 1A and B). The midgut sharply widened at the middle of the first ventriculus (≈0.5 mm) and stained a lighter blue. A bluntly ending diverticulum was found in this region. The distal end of the first ventriculus widened to a uniform diameter (0.6 mm), and then narrowed, forming an angled junction with the second ventriculus.

The second ventriculus (m_2 in Yanai and Iga 1956) was short (≈0.9 mm), looked transparent, stained dark blue, and ascended toward the thorax (Fig. 1A and B). The posterior region of the second ventriculus narrowed, turned dorsally and connected directly into

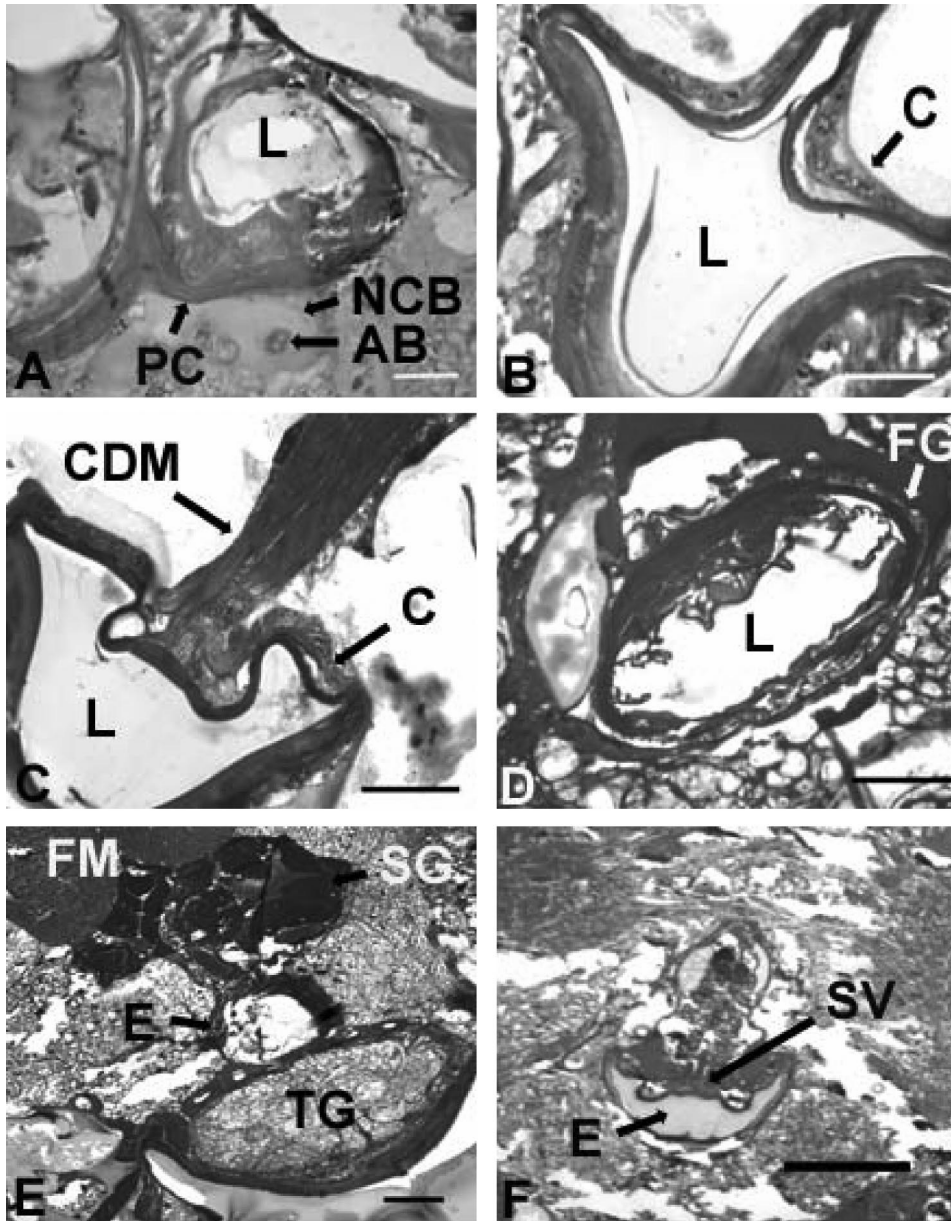

Fig. 2. Bright-field, toluidine blue-stained images of semithin transverse sections of the foregut of a female *L. hesperus*. (A) Precibarium. Scale bar = 20 μm. (B) Cibarium. Scale bar = 20 μm. (C) Thick cuticular wall of the cibarium and the cibarial diaphragm with its dilator muscle attached to dorsal part of the cibarium. Scale bar = 20 μm. (D) Esophagus with a thick cuticular wall lined with a cuticular intima. Scale bar = 20 μm. (E) Posterior esophagus, showing thinning of cuticular layer. Scale bar = 100 μm. (F) Stomodeal valve. Scale bar = 100 μm. AB, axon body; C, ciborium; CDM, cibarial dilator muscles; E, esophagus; FG, foregut; FM, flight muscle; L, lumen; NCB, neuron cell body; PC, precibarium; SG, salivary gland; SV, stomodeal valve; TG, thoracic ganglion.

the opening of the third ventriculus at the junction of the thorax and abdomen.

The third ventriculus (m_3 in Yanai and Iga 1956) had a straight cylindrical shape (≈1 mm in length), was oriented toward the end of the abdomen, looked opaque, and stained a dark blue (Fig. 1A and B). The distal end of the third ventriculus narrowed and angled dorsally, forming a juncture to the hindgut.

Hindgut. The hindgut looked transparent, was circular in cross-sectional shape and tapered from a diameter of 0.8 to 0.2 mm. The anterior hindgut cross-section was oval, 1.2 by 1 mm in diameter, and filled the body cavity of the insect (Fig. 1A). The Malpighian tubules looked green, and they were beaded and coiled. Two tubules joined dorsally and two joined ventrally with the anterior region of the hindgut (Fig.

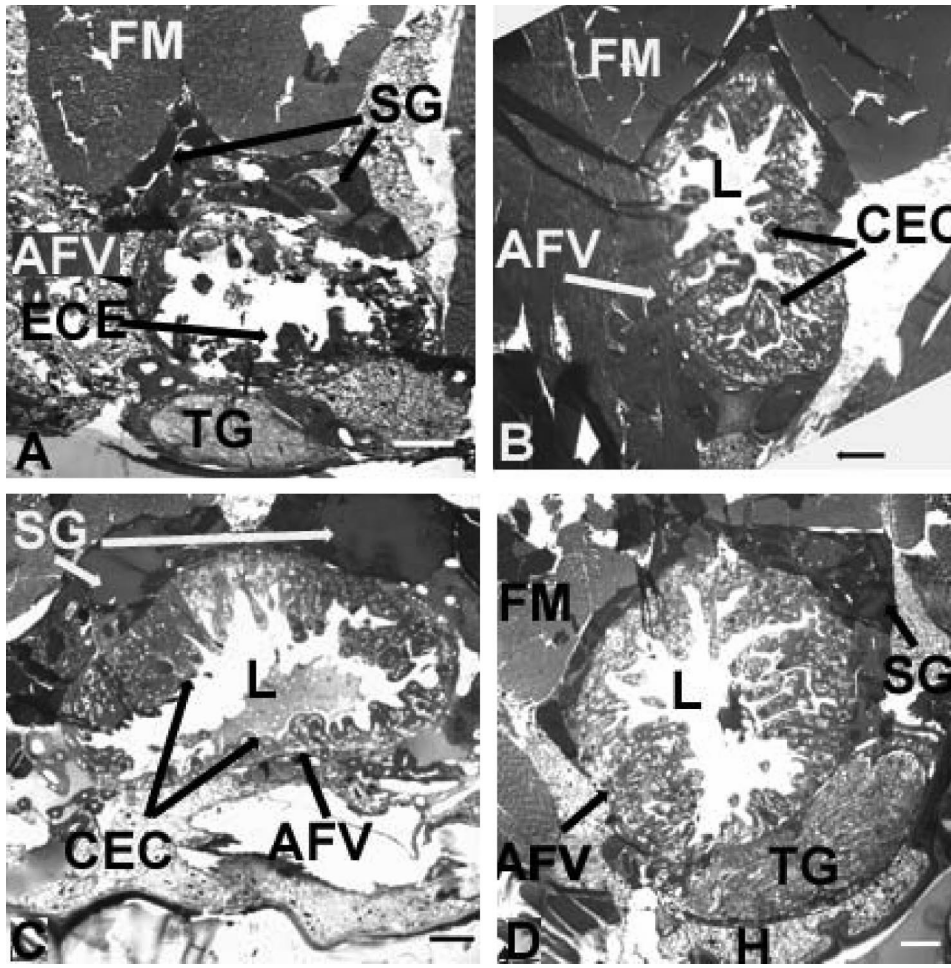

Fig. 3. Bright-field, toluidine blue-stained images of semithin transverse sections of the first ventriculus of the female *L. hesperus*. A to D progress anterior to posterior. (A) Anterior-most region of the first ventriculus, showing a single layer of intermittent epithelial cells within the open lumen. Scale bar = 100 μm. (B) Section slightly posteriad to A, showing an oval-shaped midgut containing long columnar epithelial cells and located between the flight muscle. Scale bar = 100 μm. (C) Kidney-shaped anterior region of the first ventriculus, with long epithelial cells in the dorsal part and short epithelial cells on the ventral part of the midgut. Scale bar = 100 μm. (D) Spherical region of the first ventriculus. Scale bar = 100 μm. AFV, anterior first ventriculus; CEC, columnar epithelial cells; ECE, endocrine cells; FM, flight muscles; H, hemolymph; L, lumen; SG, salivary glands; TG, thoracic ganglion.

1B). The middle region of the hindgut was narrowed (0.4 mm), pear-shaped in transverse section and located above the ovipositor. The posterior region of the hindgut, the rectum, opened into the ovipositor cavity immediately adjacent to the ovipositor (Fig. 1A and B).

Cellular Anatomy of the Alimentary Canal. Three main types of epithelial cells were found throughout the midgut of *L. hesperus:* 1) nondifferentiated, putative regenerative cells, 2) putative columnar (digestive) cells, and 3) putative endocrine cells. These categories are not fully definitive, because transmission electron microscope (TEM), immunocytochemical studies, or both have not been performed (Billingsley and Lehane 1996). However, such studies would primarily support conclusions on function, e.g., by examining basal laminal infoldings or endoplasmic reticulum of the putative columnar cells, or identify actual hormones secreted by the putative endocrine cells. We propose that our high-resolution, high-magnification, thin sections for LM by using staining similar to TEM, are sufficient to assign anatomical categories. In all cases, the shapes and sizes of these cells, the staining characteristics, and types of vesicles in the cells are nearly identical to those of the cell types described via TEM in Billingsley and Lehane (1996) and Lehane (1998).

As in most insects (Lehane 1998), the most common cell type was the columnar (digestive) cell. These cells were structurally differentiated according to the region of the gut, i.e., the shape, size, number and type of vesicles, and length and abundance of brush-border

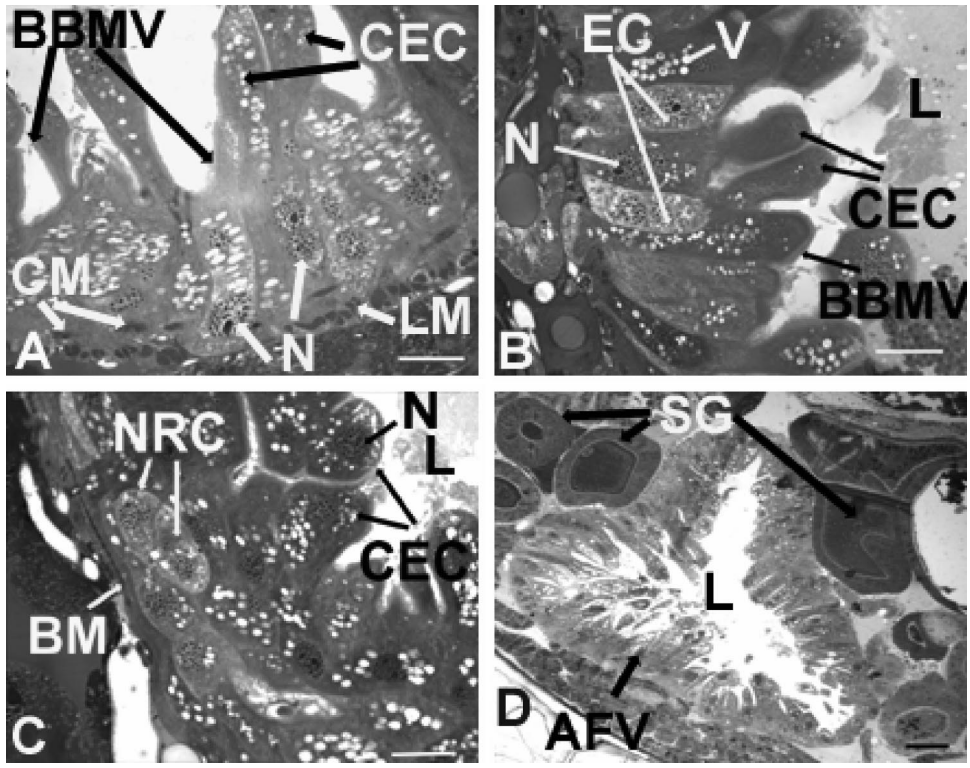

Fig. 4. Bright-field, toluidine blue-stained images of semithin transverse sections of the anterior (A–C) and middle (D) regions of the first ventriculus of the female *L. hesperus*. (A) Long columnar epithelial cells with prominent nuclei located throughout the cells and longitudinal and circular muscle under the basement membrane. Scale bar = 20 μm. (B) Pale staining of putative endocrine epithelial cells with prominent nuclei, and darker staining columnar epithelial cells with short brush-border microvilli containing transparent vesicles. Scale bar = 20 μm. (C) Pale staining, nondifferentiated regenerative cells with prominent nuclei, and darker staining columnar epithelial cells containing numerous transparent vesicles. Scale bar = 20 μm. (D) Triangular-shaped cross-section of middle region of first ventriculus, with long columnar epithelial cells. Scale bar = 100 μm. AFV, anterior first ventriculus; BBMV, brush-border microvilli; BM, basement membrane; CEC, columnar epithelial cells; CM, circular muscle; EC, endocrine cells; L, lumen; LM, longitudinal muscles; N, nuclei; NRC, nondifferentiated regenerative cells; SG, salivary glands; V, vesicles.

microvilli (BBMV) varied within each region, especially in the midgut. The columnar cells contained either medium-to-large, transparent (presumably lipid-filled; Lehane 1998) vesicles; small-to-medium, densely opaque, granular (presumably secretory; Lehane 1998) vesicles, or both. The numbers, sizes, and proportions of each type of vesicles varied somewhat with region. In contrast, the endocrine cells were shorter than columnar cells. They had small, usually numerous, opaque granular vesicles, and very few (if any), small, transparent vesicles.

First Ventriculus. Throughout the first ventriculus, well-developed inner circular and outer longitudinal muscles were observed under the basement membrane. Peristaltic movement was anecdotally observed in freshly dissected guts, in this same area. Although the cross-sectional shape of the anterior region of the first ventriculus is likely to change according to the filling state of the gut, or gut movements, in our sections it progressed from an elongated oval shape (Fig. 3A) (0.5 by 0.3 mm in diameter) containing a few short epithelial cells without BBMV, to an oval shape (Fig. 3B) (0.74 by 0.37 mm in diameter) containing long epithelial cells with very short BBMV, to a kidney shape (Fig. 3C) (0.9 by 0.45 mm in diameter), containing epithelial cells with short BBMV.

The epithelium in the anterior region of the first ventriculus consisted of the two main cell types, i.e., putative endocrine and columnar cells, intermixed. Both cell types became larger with progression posteriorly within the anterior first ventriculus. The columnar epithelial cells were finger-like in shape (74 by 12.5 μm, progressing to 188 by 16 μm in diameter) and contained medium-sized, granular vesicles or large, lightly staining vesicles in their apical regions (Fig. 4A), or both. Those columnar cells containing only transparent vesicles often seemed to have two nuclei and very short BBMV (Fig. 4A and B), although TEM studies should be performed to confirm this. About one third of the basi-lateral part of each cell was in proximity to its neighboring cell, with two thirds of each cell (termed the apical region) independently extending into the lumen of the gut (Fig. 4A). BBMV within the apical region of these cells were short and

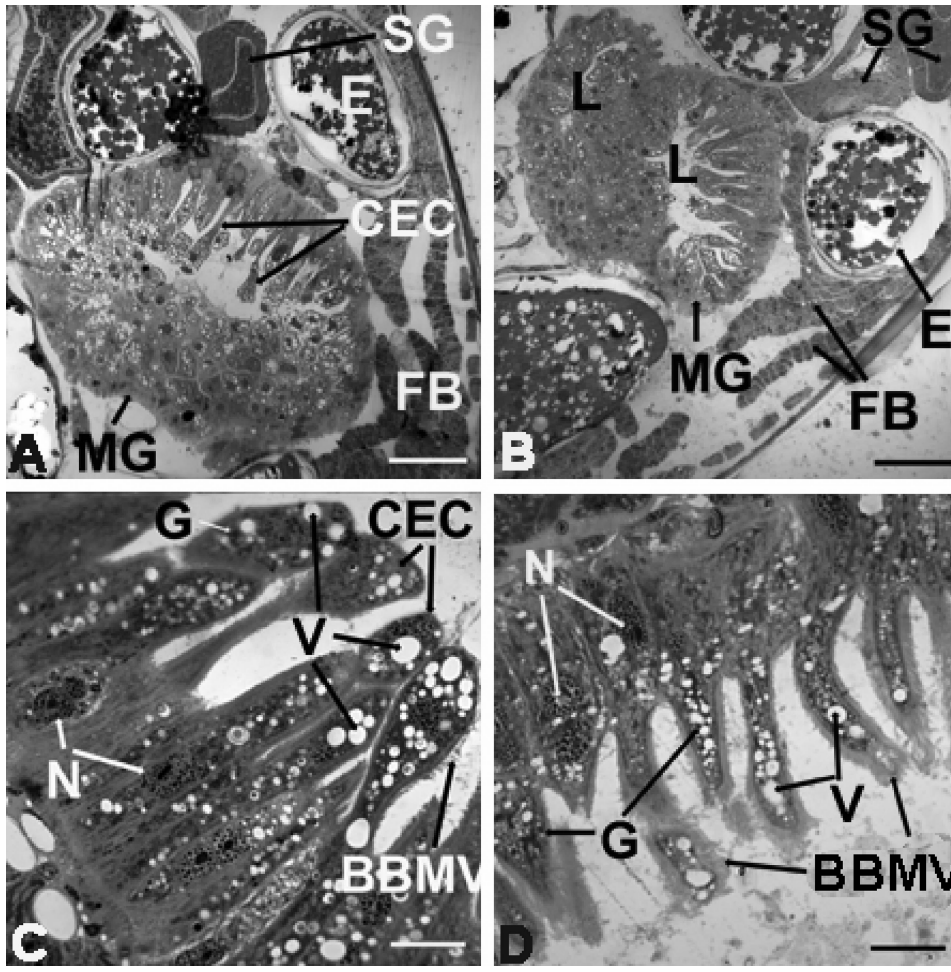

Fig. 5. Bright-field, toluidine blue-stained images of semithin transverse sections of the middle (A–C) and posterior (D) regions of the first ventriculus of a female *L. hesperus*. (A) Diverticulum area in the middle region. Scale bar = 100 μm. (B) Diverticulum, showing an additional narrow lumen. Scale bar = 100 μm. (C) Middle region showing long columnar epithelial cells with short BBMV. Scale bar = 20 μm. (D) Posterior region showing long columnar epithelial cells with long BBMV. Scale bar = 20 μm. BBMV, brush-border microvilli; CEC; columnar epithelial cells; E; egg; FB; fat body; G; granular vesicles; L; lumen; MG; midgut; N; nuclei; SG; salivary glands; V; transparent vesicles.

sparse at the blunt end and became more numerous in the unattached lateral regions (Fig. 4B and C). Some nuclei within the columnar cells with only transparent vesicles were located basally, close to the basement membrane, whereas other cells had nuclei located apically (Fig. 4B and C).

Interspersed among the columnar cells were putative endocrine cells. These were shorter than the columnar cells, and their cytoplasm stained much more lightly. These cells had numerous small, opaque granular vesicles located in their medial-to-basal regions, and only a few transparent vesicles, which were usually smaller than those in the columnar cells. These cells were closed endocrine cells (Endo and Nishiitsutsuji-Uwo 1981); serial sections showed that their apical margins did not border the gut lumen; thus they were without BBMV. Usually oval in shape (≈48 by 12.5 μm in diameters), these cells seemed to contain two nuclei each (Fig. 4B). One endocrine cell usually occurred between every two to four columnar cells. These closed endocrine cells were observed primarily in the anterior part of the first ventriculus, with only a few, scattered cells observed elsewhere.

Immature, apparently nondifferentiated cells also were found in the anterior region of the first ventriculus, in proximity to the basement membrane. These cells were oval (27 by 12 μm in diameter) and seemed to contain one or two nuclei (Fig. 4C). In the oblique orientation of Fig. 4C, their long axes looked parallel to the basement membrane. These cells stained much lighter than the neighboring epithelial cells (Fig. 4C). This description matches that of regenerative cells (Billingsley and Lehane 1996). The number of these regenerative cells in this region was much greater than in other regions of the midgut.

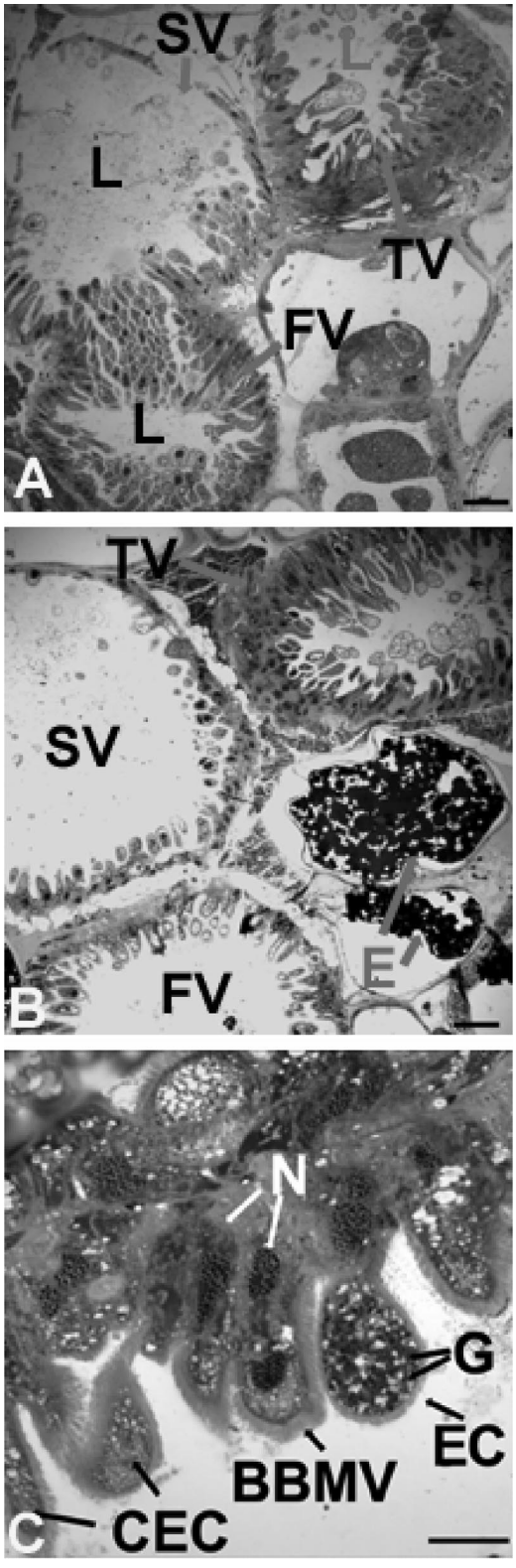

Fig. 6. Bright-field, toluidine blue-stained images of semithin transverse sections of the first, second, and third ventriculi of a female *L. hesperus*. (A) Area at the junction of the first and second ventriculi showing all three ventriculi. Scale bar = 100 μm. (B) The three ventriculi of the midgut. Scale bar = 100 μm. (C) The second ventriculus at higher magnification showing short columnar epithelial cells. Scale bar = 20 μm. BBMV, brush-border microvilli; E, egg; FV, first ventriculus; G, granules; L, lumen; L-CEC, lipoid columnar epithelial cells; N, nuclei; O-CEC, opaque columnar epithelial cells; SV, second ventriculus; TV, third ventriculus.

The middle region of the first ventriculus was oriented ventrally in the hemocoel. The cross-sectional shape of the anterior-most part of this middle region was triangular (Fig. 4D) and contained long columnar epithelial cells (≈140–180 μm) ≈1.5–2 μm). These cells contained numerous small vesicles, and the nuclei were randomly located within the cells (image not shown). At the center of the middle region (Fig. 5A), the epithelium was folded, forming a small diverticulum. Thus, cells in that region were cross-sectioned rather than longitudinally sectioned, seeming to clump into the lumen of the gut in a seemingly multilayered manner (Fig. 5B). In this region, the epithelium on the opposite side of the gut consisted of single-layered, long columnar cells (≈110 μm) with short BBMV (≈1.5–2 μm) (Fig. 5C). In contrast, the epithelium of the folded area consisted of short columnar cells without BBMV. Columnar cells on all sides of the lumen contained a mixture of granular and transparent vesicles (image not shown), as in the anterior region of the first ventriculus. No endocrine cells were observed in this region.

In the posterior portion of the first ventriculus, the cross-sectional shape of the gut narrowed to ≈0.5 mm, and the lumen became smaller. The epithelium consisted of long finger-like columnar cells (≈95 μm) with very long BBMV (≈4–8 μm; Fig. 5D). These cells contained numerous lightly staining vesicles, and a few opaque, granular vesicles in their apical regions, but fewer than in the previous portion of the first ventriculus. The nuclei were primarily located basally, where the cells were in proximity with neighboring cells (Fig. 5D). The distal end of the first ventriculus narrowed before it formed an angled juncture to the second ventriculus (Fig. 6A).

Second Ventriculus. The second ventriculus was oriented toward the thorax, and it was located dorsad to the first ventriculus within the hemocoel. A transverse section of the second ventriculus was circular in shape, and wider (0.9 mm in width) (Fig. 6B) and shorter (1 mm in length) (Fig. 1B) in comparison to the first ventriculus. Columnar cells in the transverse sections of the entire second ventriculus were shorter (≈50–60 μm) than those in the first ventriculus and had (apparently) two prominent nuclei per cell located close to the basement membrane (Fig. 6C). Two distinctly different columnar cells were observed. The first was a short cell (≈50 μm in length), with only a few transparent vesicles and having long BBMV (≈3.5–4 μm in length) (Fig. 6C). The second cell type was filled with many medium-sized granular vesicles and also contained a few small, transparent vesicles

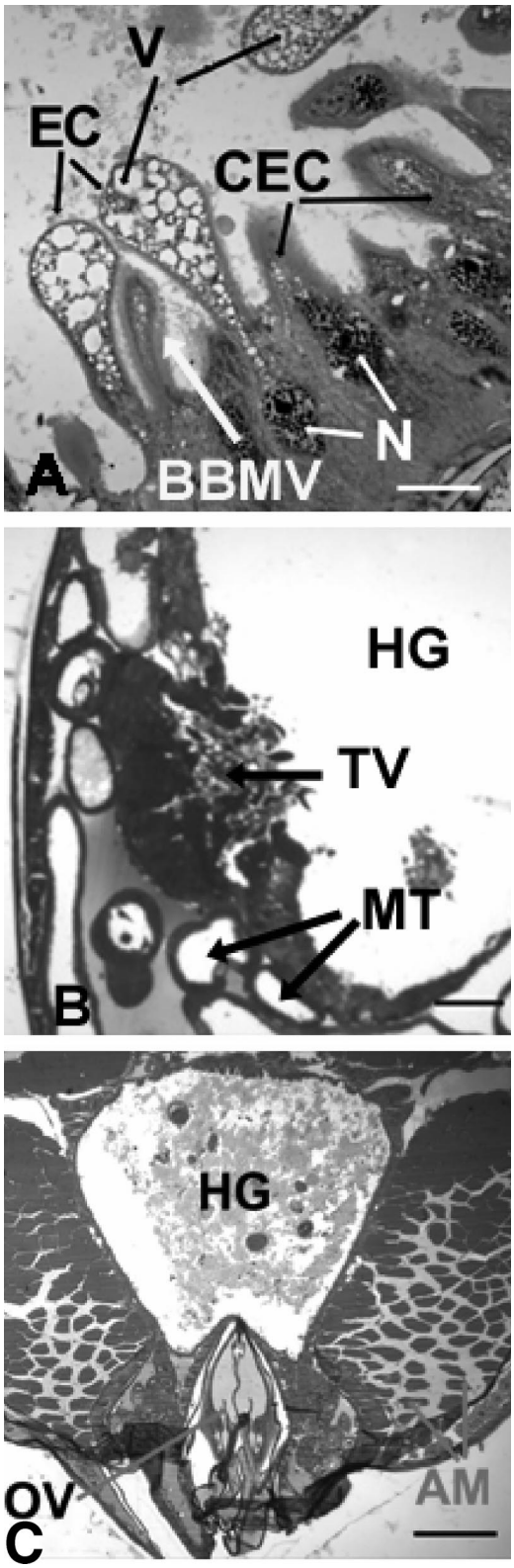

Fig. 7. Bright-field, toluidine blue-stained images of semithin transverse sections of the third ventriculus, hindgut, and the Malpighian tubules of the female *L. hesperus*. (A) Third ventriculus showing short columnar epithelial cells with long brush-border microvilli, long droplet-shaped, vesiculated endocrine epithelial cells with very short BBMV. Scale bar = 20 μm. (B) Area where the third ventriculus joins the hindgut. Scale bar = 100 μm. (C) Hindgut showing extremely thin epithelial layer with a large lumen. Scale bar = 100 μm. AM, abdominal muscle; BBMV, brush-border microvilli; HG, hindgut; L, lumen; L-CEC, lipoid columnar epithelial cells; MT, Malpighian tubules; N, nuclei; O-CEC, opaque columnar epithelial cells; OV, ovipositor; V, vesicle.

(Fig. 6C). It was a short cell (≈40 μm in length) that was bulbous-shaped with short BBMV (≈1.5 μm). Cells with transparent vesicles were more abundant than cells with granular vesicles, but both cell appearances were intermixed and distributed throughout the entire length of the second ventriculus.

Third Ventriculus. The cross-sectional shape of the third ventriculus appeared uniform and oval (≈0.8 by 0.45 mm in diameter) (Fig. 6B). It was again composed of two types of columnar cells (Fig. 7A). Both were very long, resulting in a thick epithelium and a narrow lumen (Fig. 6B), with prominent, oval-shaped nuclei that were located either medially or basally, i.e., close to the basement membrane. The first cells were finger-like (≈40–60 μm in length and 7–10 μm in width) with long BBMV (≈3.4 μm in length) and few transparent vesicles (Fig. 7A). The other cells were very long and narrow (≈95–100 μm in length and 18–20 μm in width) and droplet-shaped. Prominent extensions into the lumen were filled with numerous, large, transparent vesicles and a few opaque, granular vesicles (Fig. 7A). These cells contained very short BBMV on the lateral part of the apical region and almost no BBMV on the rounded end of the cell (Fig. 7A). The abundance of the droplet-shaped cells in the third ventriculus was very high, and in some areas the epithelium consisted only of this cell type (Fig. 6B). In contrast to the first ventriculus, very limited circular or longitudinal muscles were observed associated with the basement membrane of the second and the third ventriculi (Fig. 7A).

Hindgut and Malpighian Tubules. Both the hindgut and the Malpighian tubules had an extremely thin epithelial cell layer and a cuticular intima that were not well resolved via the LM used for this study (Fig. 7B and C).

Discussion

Comparative Gross Morphology of the Hemipteran Gut. Overall, our work shows that the alimentary canal of *L. hesperus* is a relatively simple, unadorned gut, compartmentalized into differently shaped regions, including one small diverticulum (not a caecum). Before our work, the sole gut anatomical studies of hemipterans from the suborder Heteroptera presented only drawings of gross morphology and occasional cellular histology, not micrographs (Miyamoto 1961; Goodchild 1963, 1966). We confirm the conclusions in this older literature, i.e., that this Lygus gut

(Yanai and Iga 1956, Miyamoto 1961) or simple, cimicomorph type (Goodchild 1966) is the least complex gut type in the order Hemiptera. We also add further information revealed by higher resolution images. This conclusion is supported by the following, comparative morphological details.

The ventral or dorsal microdissections of adult female *L. hesperus* showed that the esophagus was very thin, similar to that of other heteropterans (Goodchild 1966) and the suborder Auchenorrhyncha (Tsai and Perrier 1993). *L. hesperus* lacks a true filter chamber, such as is found in the "Cicadoidea" type alimentary canal of today's superfamilies Membracoidea and Cicadoidea (Auchenorrhyncha) (Goodchild 1966, Cheung and Purcell 1993) or in the "Coccoidea" type (Goodchild 1966). Likewise, *L. hesperus* also lacks a pseudofilter chamber, i.e., an engulfing sheath, present in the "Fulgoroidea" type (Goodchild 1966, presently infraorder Fulgoromorpha), which is thought to function much as a filter chamber. There is also no other specialized structure such as the stomodeal crop or proventriculus found in Aleyrodidae (Sternorrhyncha) (Ghanim et al. 2001). In addition, a large, gastric caeca-bearing area was not observed in *L. hesperus*, although a small, blunt, sac-like diverticulum was observed in the middle of the first ventriculus. Such a caecum-like structure is common in the alimentary canal of Pentatomidae (infra-order Pentatomomorpha). In contrast, diverticula are not well described in the infra-order Cimicomorpha, which contains *L. hesperus.*

The literature differs on how many segments occur in the simple, cimicomorphan midgut. Like us, Yanai and Iga (1956) found that the *Lygus*-type gut's key characteristic was three segments and no gastric caecae. In contrast, although Goodchild (1963) referred to the Yanai and Iga (1956) *Lygus* gut type, he included it under his general Cimicomoph gut type and referred to that broader type as having two-segmented midguts. Evidently, the *Lygus*-type gut does not extend to all mirids, because the representative mirid of Miyamoto (1961) also has a two-segmented midgut. Thus, cimicomorphans may have two or three segments.

Comparative Cellular Anatomy of the Hemipteran Gut. In contrast to the overall morphology of the gut, epithelial cellular anatomy was relatively complex and similar to other insects, with three different cell types present (two mature and one immature). Although neither TEM nor immunocytochemistry was performed for this study, evidence supports that the two mature cell types are endocrine and columnar epithelial cells. This evidence consists primarily of several TEM and immunocytochemical studies of closely related hemipterans (Sehnal and Zitnan 1996), showing nearly identical-looking cells in the same general locations as in *L. hesperus.* Endocrine and columnar cells are also the primary epithelial cell types in most insect guts (Lehane 1998). Within columnar epithelial cells, there were two distinctly different appearances (opaque, granular versus transparent vesicles) that may represent different functional or physiological states of a single, pleomorphic, columnar cell type. These conclusions are supported by the following detailed observations.

The midgut's first ventriculus had large circular and longitudinal muscles under the basement membrane that were not as well developed in the second and third ventriculi. This corresponds with our anecdotal observation of rapid and frequent peristalsis proceeding from anterior to posterior in the first ventriculus, but it was not observed in the second and third ventriculi. However, this differs from the poorly developed inner circular and outer longitudinal muscles in the midgut of heteropteran salivary sheath feeders (Pentatomomorpha) (Billingsley and Lehane 1996).

Two other epithelial cell types were observed in the midgut of *L. hesperus.* The first, closed endocrine cells (Endo and Nishiitsutsuji-Uwo 1981), were found almost exclusively in the anterior region of the first ventriculus in *L. hesperus.* This location is similar to findings with cicadas (Auchenorrhyncha: Cicadomorpha: Cicadoidea), and *Dysdercus cingulatus* (F.) and *Oncopeltus fasciatus* (Dallas), two pentatomomorph species of Heteroptera (Zitnan et al. 1993, Sehnal and Zitnan 1996), whose endocrine cells are in the anterior midgut. This location of endocrine cells is quite different from that of *Rhodnius prolixus* Stål, a blood-sucking cimicomorphan heteropteran, whose endocrine cells are in the posterior midgut (Sehnal and Zitnan 1996).

The second type of epithelial cell in *L. hesperus*, columnar cells, was found throughout the midgut. These cells had both densely opaque, granular vesicles, and medium-to-large transparent vesicles. Sometimes, these two types of vesicles were mixed together in one cell, but more often one type predominated over the other, correlated with size, shape, length of BBMV, and location in the gut.

The columnar epithelial cells match the description (without illustration) of the two cell types of Goodchild (1963). That author stated that one type was seen throughout the gut. It had narrow bases with bulbous tips that project into the lumen, the cytoplasm was usually full of basophile granules, and there were one large, or several small vacuoles. These cells develop a tall, narrow, shape as they mature. This description matches the cells seen in our Fig. 5. The second cell type of Goodchild (1963) was seen primarily in the anterior first ventriculus. These cells have an approximately cuboid cell base and a large lobe that projects into the lumen. The junction of the lobe with the base is distinctly constricted. Also, the cytoplasm is always dense, finely granular, and without vacuoles. This description matches the cells seen in our Fig. 4. Therefore, we confirm the observations of Goodchild (1963) observations, and we also provide more information on cellular fine structure, such as the BBMV.

Past TEM and immunocytochemical studies of columnar cells from several insect species show that darkly staining, granular vesicles are associated with a secretory function (termed secretory vesicles), whereas the large, lightly staining vesicles (termed

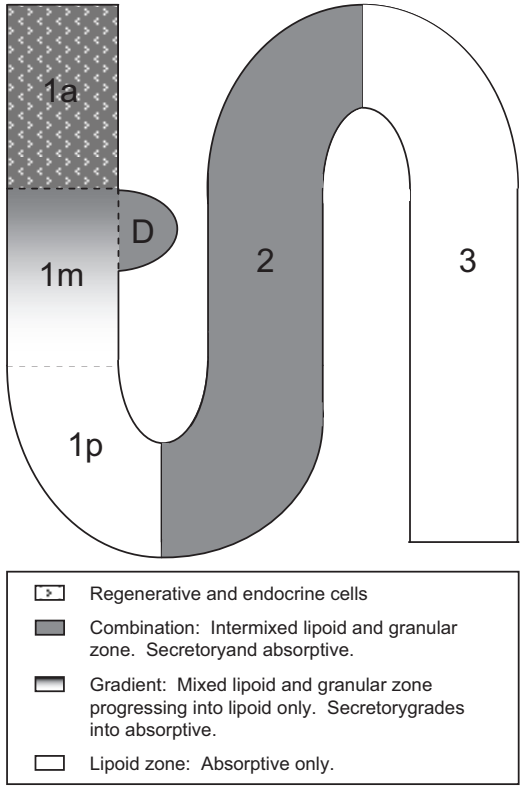

Fig. 8. Diagrammatic representation of cellular differentiation in the midgut of *Lygus hesperus.* 1, 2, and 3, first, second, and third ventriculi of the midgut, respectively. a, anterior; m, middle; p, posterior; and D, diverticulum .

lipid vesicles) are associated with absorption as the function of that cell (Billingsley and Lehane 1996, Lehane 1998). Our relatively high-resolution LM images of semithin sections show striking resemblances to the TEM images therein. Therefore, we propose that similar functions occur in *L. hesperus* cells. Those cells with mixed granular and vesicular appearance may function in both manners. These interpretations will need to be verified by higher resolution TEM-level studies and morphometric analysis.

Differentiation of Cellular Anatomy into Functional Zones. We found that the *L. hesperus* midgut is structurally differentiated by cell type along its length, as is the midgut of most insects (Terra et al. 1996). For example, the midgut of the blood-sucking stable fly, *Stomoxys calcitrans* (L.), has several distinct zones. Two *S. calcitrans* zones that seem similar to *L. hesperus* zones are the 1) opaque zone, in which columnar cells with secretory vesicles predominate and digestive enzymes are secreted (Lehane 1976, Billingsly and Lehane 1996); and 2) lipoid zone, in which columnar cells with lipid vesicles predominate, and where digestion proceeds and absorption takes place (Lehane 1977, Billingsly and Lehane 1996). Cell type zones for *L. hesperus* are diagrammed in Fig. 8. The anterior area of the first ventriculus houses nearly all of the regenerative and endocrine cells, and the columnar cells therein have mixed granular and transparent vesicles (i.e., mixed opaque and lipoid zone). In the middle to posterior areas of the first ventriculus, columnar cells gradually progress toward predominantly transparent vesicles (i.e., lipoid). The second ventriculus reverts to a mixture of vesicle types, each within its own cells and also with cells containing both types (i.e., opaque and lipoid). Cells with transparent vesicles then predominate again in the third ventriculus (i.e., lipoid) (Fig. 8).

Assuming that *L. hesperus* midgut cells function in the same ways as other insects' cells with similar anatomical features, then the first ventriculus may exhibit a functional gradient. Mostly regulatory/regenerative cells occur in the anterior portion, a mixture of secretory and absorptive cells occurs in the middle portion, gradually increasing in absorptive function into the posterior portion of the first ventriculus. Secretory and absorptive functions are then mixed again in the second ventriculus, whereas the absorptive function is most important again in the third ventriculus.

These general functions of midgut cells are supported by some of our previously published observations of ligand binding and cellular disruption by nutrient and non-nutrient proteins fed per os to *L. hesperus.* For example, phytohemagglutinin, a lectin from red kidney bean, *Vicia faba* L., binds to the BBMV and is endocytosed by epithelial cells of second instars of *L. hesperus* throughout the midgut. However, the most severe damage by this lectin occurs at the anterior and posterior first ventriculus and in the third ventriculus (Habibi et al. 2000). These are zones where the lipoid (probably absorptive) columnar cells predominate. The varying degrees of damage might be due to different densities of GalNAc-lectin binding receptors, which plausibly could be concentrated on the most absorptive cell types. Other studies have shown that green fluorescent protein (Habibi et al. 2002) and Cry2Ab (Brandt et al. 2004) also bind to the BBMV of the midgut epithelial cells of adult females.

Role of Phylogeny versus Feeding Strategy in Evolution of the Hemipteran Gut. Previous studies have shown that the relationships among gross morphology, cellular anatomy, and digestive function in insect guts correlate better with phylogeny than with type of food, per se (Goodchild 1963, Terra et al. 1996). Although our work generally supports this conclusion, our more detailed anatomical studies suggest a refinement. We propose that the gross morphological features of the hemipteran gut are strongly correlated with the feeding strategy of the species of interest, which, in turn, has been shown to be highly correlated with the phylogeny and subordinal classification of Hemiptera. However, the cellular anatomy and some functions of gut cells seem to be correlated both with feeding strategy and food type. Cell type distribution is correlated with food type. The following details support this conclusion.

L. hesperus, like all cimicomorphan heteropterans, is a cell rupture feeder (Backus et al. 2005) (formerly termed a lacerate-and-flush feeder, Miles 1972, 1999;

Taylor and Miles 1994) that ingests a semiviscous slurry of partially (extraorally) digested cell contents from a variety of plant cell types (Backus et al. 2007). Thus, it ingests a relatively low volume of nutritionally complete fluid, during discrete meals or feeding bouts. In contrast, most other hemipterans use the salivary sheath-feeding strategy (Miles 1972). This includes all insects in the suborders Sternorrhyncha and Auchenorrhyncha [except the cicadellid subfamily Typhlocybinae, including *Empoasca fabae* (Harris), which, like *L. hesperus*, is a cell rupture-feeder; Backus et al. 2005], and the heteropteran infra-order Pentatomomorpha.

Sheath-feeders continuously ingest an ultimately much higher volume of nutritionally incomplete phloem or xylem sap, compared with cell rupture-feeders. Most have evolved some means of handling high volumes of dilute sap food. Aphids have a short, straight, tube-like alimentary canal, allowing very rapid gut passage of dilute food. Most other sheath feeders have longer guts with elaborate filter chambers or pseudofilter chambers, to concentrate the nutrients by shunting water straight to the Malpighian tubules and hindgut (Goodchild 1963, 1966). They also use the specialized caecae/diverticula to further maintain water balance between the gut lumen and the hemolymph (Goodchild 1966, Dow 1986). Caecae in the Pentatomomorpha also may house symbiotic microbes, as in other insects (Douglas and Beard 1996).

The alimentary canal of *L. hesperus* has several features that are very different from those of salivary sheath feeders (such as a short foregut, three ventriculi with five subdivisions, no caecum, and a long hindgut). Cell rupture-feeders, whether plant feeding such as *L. hesperus* and *E. fabae*, or blood feeding such a *R. prolixus*, had no need to evolve elaborate filter chambers and other morphological features to handle continuous ingestion of dilute fluid. Therefore, it is not surprising that their gross gut morphology is simpler than that of sheath-feeders. Yet, cellular anatomy and function, especially distribution of endocrine cells, is different between the plant-feeder *L. hesperus* and the blood-feeder *R. prolixus* (Sehnal and Zitnan 1996). Cellular anatomy is likely to be correlated with the physiology of digestion and absorption, which are very likely to differ between plant sap versus blood. Therefore, phylogeny and feeding strategy seem to be correlated with both gross gut morphology and some aspects of cellular anatomy. However, physiological adaptations to food type correlate with distribution of cell types and how that impacts their functions.

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